

CLAIMS

a 1. Method for producing <sup>the</sup> ~~a~~ recombinant protein of interest whose gene is placed under the control of the P<sub>trp</sub> tryptophan operon promoter, characterized in that it comprises the following steps:

- a1 > 4/2/9
- a) transforming a prokaryotic cell with a vector containing a nucleic acid sequence which is capable of inactivating the gene encoding a TnaA tryptophanase when said nucleic acid sequence is introduced into said host cell, and integrating said sequence into the DNA of said host cell; and beforehand, subsequently or simultaneously, introducing into said prokaryotic cell all, or part, of the sequence of a promoter which is followed, in the 3' position, by a nucleic acid sequence encoding a molecule which is ribonucleotide or protein in nature, and which acts negatively on the P<sub>trp</sub> promoter or its transcription product;
- b) transforming said prokaryotic cell with a vector containing a gene encoding said recombinant protein of interest;
- c) culturing said transformed cell in a culture medium which allows the expression of the recombinant protein; and
- d) recovering the recombinant protein from the culture medium or from said transformed cell.

Sub B' 2. Method for producing a recombinant protein of interest ~~according to Claim 1~~, in which the nucleic acid sequence which is capable of inactivating the gene encoding a TnaA tryptophanase is introduced into the DNA of the prokaryotic host cell according to the chromosomal integration method described in Example 1 or 2.

a 3. Method for producing <sup>the</sup> ~~a~~ recombinant protein of interest ~~according to either of Claims 1 and 2~~, in which said nucleic acid sequence introduced into said

host cell is introduced without any other DNA element which would allow a selective advantage to be associated therewith.

a 4. Method for producing a recombinant protein of ~~interest according to one of Claims 1 to 3,~~ in which said nucleic acid introduced into said host cell is introduced at the tryptophanase operon locus.

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a 5. Method for producing a recombinant protein of ~~interest according to one of Claims 1 to 4,~~ ~~wherein~~ characterized in that said method also comprises, between step a) and b), a resolution and a screening step.

Sub B2 6. Method for producing a recombinant protein of ~~interest according to one of Claims 1 to 5,~~ in which the induction of said promoter which is followed, in the 3' position, by a nucleic acid sequence encoding a molecule which is ribonucleotide or protein in nature, and which acts negatively on the Ptrp promoter or its transcription product is obtained by any means enabling an inhibitory or activating effect to be exerted on said promoter.

a 7. Production method ~~according to~~ Claim 6, in which the induction of said promoter which is followed, in the 3' position, by a nucleic acid sequence encoding a molecule which is ribonucleotide or protein in nature, and which acts negatively on the Ptrp promoter or its transcription product, is obtained either:

- a) by choosing a suitable carbon source in the culture medium; or  
b) by adding tryptophan to the culture medium; or  
c) by a combination of a) and b).

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a 8. ~~Method according to one of Claims 1 to 7,~~ ~~wherein~~ characterized in that the prokaryotic host cell is a Gram-negative bacterium.

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a 9. ~~Method according to one of Claims 1 to 8,~~ ~~wherein~~ characterized in that the prokaryotic host cell is *E. coli*.

Sub  
B3

10. First construct for transforming a prokaryotic host cell which can be transformed with a second construct for expressing a gene encoding a recombinant protein ~~of interest~~ placed under the control of the P<sub>trp</sub> tryptophan operon promoter in a prokaryotic host cell, ~~characterized in that~~ <sup>wherein</sup> the first construct comprises a nucleic acid sequence which is capable of inactivating the gene encoding a TnaA tryptophanase when said nucleic acid sequence is introduced into said host cell.

11. First construct ~~according to~~ <sup>of</sup> Claim 10, characterized in that it also comprises, upstream of said nucleic acid sequence capable of inactivating the gene encoding a TnaA tryptophanase when said nucleic acid sequence is introduced into said host cell, all or part of the nucleic acid sequence of the P<sub>tna</sub> tryptophanase operon promoter.

12. First construct ~~according to either of Claims 10 and 11,~~ <sup>wherein</sup> ~~characterized in that~~ said nucleic acid sequence capable of inactivating the gene encoding a TnaA tryptophanase when said nucleic acid sequence is introduced into said host cell comprises a mutated fragment of the coding sequence of said TnaA tryptophanase.

13. First construct ~~according to~~ <sup>of</sup> Claim 12, ~~characterized in that~~ <sup>wherein</sup> said mutated fragment is obtained by inserting a stop codon at a position such that the sequence of the mutated fragment thus obtained encodes a protein fragment lacking tryptophanase activity.

14. First construct ~~according to either of Claims 12 and 13,~~ <sup>wherein</sup> ~~characterized in that~~ said mutated fragment is a mutated fragment of the coding sequence of the TnaA tryptophanase of said host cell.

15. First construct ~~according to~~ <sup>of</sup> Claim 10, ~~characterized in that~~ <sup>wherein</sup> said nucleic acid sequence capable of inactivating the gene encoding a TnaA tryptophanase when said nucleic acid sequence is introduced into said host cell is the nucleic acid

sequence comprising all or part of the sequence of a promoter followed, in the 3' position, by a nucleic acid sequence encoding a molecule which is ribonucleotide or protein in nature, and which acts negatively on the Ptrp promoter or its transcription product.

16. First construct ~~according to~~ <sup>of</sup> Claim 15, ~~characterized in that~~ <sup>wherein</sup> said promoter followed, in the 3' position, by a nucleic acid sequence encoding a molecule which is ribonucleotide or protein in nature, and which acts negatively on the Ptrp promoter, is all, or a part permitting promoter activity, of the Ptna tryptophanase operon promoter.

17. First construct ~~according to~~ <sup>of</sup> Claim 16, ~~characterized in that~~ <sup>wherein</sup> said nucleic acid sequence encoding a molecule which is ribonucleotide or protein in nature, and which acts negatively on the Ptrp promoter, is the sequence encoding the TrpR tryptophan operon aporepressor or one of its biologically active fragments.

18. Vector containing a first construct ~~according to one of Claims 10 to 17.~~ <sup>of</sup>

19. Vector ~~according to~~ <sup>of</sup> Claim 18, ~~characterized in that~~ <sup>wherein</sup> it is the vector pMAK705 [tnaAt] ~~as defined in Example 1~~ or the vector pMAK705 [Ptna :trpR :3'tna].

20. Prokaryotic host cell transformed with a vector ~~according to either of Claims 18 and 19.~~ <sup>of</sup>

21. Prokaryotic host cell ~~according to~~ <sup>of</sup> Claim 20, ~~characterized in that~~ <sup>wherein</sup> it is E. coli.